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CONFIDENTIAL FACSIMILE TRANSMISSION

DATE: 6/27/03

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FROM: Joe Aceto

Pages:
including cover: 10

Message

Please find a rough draft & with the issues discussed in the June 17 office interview.

Thank you

Confirmation Copy of email

CONFIRMATION

RE: US application 09/801,471

Dear Dr. Canella:

Unfortunately you were ill on June 17, and we were not able to meet with you for our scheduled interview at the PTO. Examiner Caputa was able to provide extensive input into the substantive issues connected with the recent Office Action, along with Dr. Liberti who flew in from New Mexico for the meeting. Present for the meeting were myself (Joe Aceto-50,701) and Jared Mayes (51,292) from Philadelphia, Jim Wilcox (30,234) from Ohio, and inventor Paul Liberti from New Mexico. In accordance with Examiner Caputa's instructions, we have provided a rough draft to encompass these suggestions which hopefully will clear up outstanding issues.

The primary issues in items 13-14-15-16-17-18-19 of the office action focus upon 35 U.S.C. 103(a) obviousness. These objections, in part, combine the prior art of Davis, Leif et al., and Maples et al. which would be motivated by the teachings of Gross et al. and Terstappen et al.

With this backdrop, Dr. Liberti and the other applicants strongly suggest that this motivation does not exist. Dr. Liberti pointed out to Dr. Caputa that the function of internal control cells of the present invention are quite different than any other control cells heretofore described, controlling for several very different variables. He described these functions as follows: (1) the cells control for magnetic labeling reagent, i.e. the integrity of the ferrofluids (2) the cells control for the integrity of the magnetic separation devices used in the process (3) the cells control for the integrity of labeling agents used following the enrichment process and (4) the control cells give the operator a good indication of the efficiency of the enrichment process and/or the detection-analysis portion of the assay. Dr. Liberti also pointed out that it is indeed a surprising result that cells can be prelabelled with membrane soluble dyes, permeablized with mild detergents, fixed and stored and yet retain membrane label through the process as well as the ability to have its internal components available for subsequent labeling at any time up to six months. He stated that permeablization reagents should have a significant effect on membrane soluble dyes (causing them to leach out) and further that such cells are known to be fragile. Yet the inventors have been able to produce such a cell. A cell that retains its prelabelling such that an

operator can have the confidence that no more than 1/10,000 cells might be unlabelled – a critical factor when one is controlling for the presence of endogenous tumor cells. In a subsequent discussion with me, Dr. Liberti mentioned that it is quite amazing that under a microscope stored control cells look no different than fresh culture cells (these are SKBR3 cells). Further in rare cell detection assays, most investigators are reluctant to spike control cells into an unknown sample that has the same morphologic and antigenic properties as the cells being detected. In the present invention, the specificity of labeling is so complete that these control cells can be safely added to a blood sample in rare cell detection analysis.

The morphologic characteristics of the control cells in Davis were preserved through freeze-drying and were subsequently labeled after re-hydrating before their use. Neither Gross et al. nor Terstappen et al. would provide a motivation to combine both the stability of the antigen-antibody complexes in leukocytes after cross-linking (Leif et al.) and the suggested improvement of differentially labeled control cells over fluorescent beads (Maples et al.) with the teachings from Davis. One reason for this is that there is no suggestion in Gross et al. to utilize multiple fluorescent entities at one or more cell specific sites (i.e. multiple fluorochromes linked to one or more antibodies for recognition at antigenic sites) in a way that would ensure detecting cell labels through a single spectral window or gate. Gross et al. relied on using multiple antibodies conjugated to a single fluorescent moiety (PerCP) to detect cytokeratins 5, 6, 8 and 18. Gross et al. was not concerned with ensuring the high level of confidence for individual cell recognition as in the redundant labeling of the present invention. Thus because a major factor suggesting the development of an internal control cell is not present, there would be no motivation to pre-label, permeabilize and then fix cells for use as an internal control.

Further, the control cells of the present application are labeled with high efficiency. Gross et al. provides for a manner to detect a low frequency of target cells, thus there would not be any suggestion for developing control cells with a high efficiency of labeling. In fact, Gross et al. uses a covalent method for labeling BT-20 cells with 7-amino-4-chloromethylcoumarin which has a low efficiency of labeling (i.e. labeling at 4°C prior to paraformaldehyde fixation; see page 235, col 2).

In order to clarify these points, Examiner Caputa suggested incorporating "distinct" into the first el ment of the claims describing the redundant labeling as shown below in the amended claims. There is support for this change within the specification (page 9 para 0093 and page 10, para 0094) as exemplified with the fluorescent labeled HER81 antibody.

Further, this incorporation would address the issue in item 6 of the office action where the use of "redundant labeling..." was considered unclear. Redundant labeling as described (page 9, para 0093) can be optionally done by concurrent or sequential addition of a second pre-label. For example, the octadecyl indocarbocyanines (DiIC18) and the octadecyl oxacarbocyanines (DiOC18) have an emission spectra of 530 to 700 nm and 450 to 650 nm, respectfully ("Handbook of Fluorescent Probes and Research Chemicals" Haugland R.P. 5th ed. 1994; 260). These are two distinct fluorescent labels possess overlapping emission spectra. Selecting a single detection gate within the overlapping spectra provides the redundant labeling to ensure internal control cell detection (page 9, para 0093).

In item 15, claim 35 is drawn to an improved method for detecting and enumerating rare cells. For the above stated reasons, utilizing multiple labels in a control cell preparation would provide the necessary motivation to use these cells as an internal control. With the high specificity of labeling in the present invention, the reduced risk of mislabeling or partially labeling, and the ability to store cells for extended periods of time, there is no motivation in the prior art to use the cells of the present invention as an internal control in rare cell detection analysis, especially where early detection of only a few rare cells might correspond to a disease state (See Example 6 page 12, para 0122).

Another issue centers on item 12 of the office action. Item 12 is based on 35 USC 112, first paragraph relating to the enablement of a control cell comprising a detectably labeled surface determinant which is an estrogen or androgen determinant. While not mentioned in the 2001 edition of Goodman & Gilman's The Pharmacological

Basis of Therapeutics, ther is ample support in the literature that supports the existence of estrogen and androgen receptors outside the nucleus.

1- "Cellular functions of plasma membrane estrogen receptors" Levin, E.R. Steroids 2002; 67; 471-475.

2- "Plasma membrane estrogen receptors signal to anti-apoptosis in breast cancer" Razandi M, Pedram A, Levin E.R. Mol Endocrinol 2000; 14; 1434-1447.

3- "An oestrogen membrane receptor participates in estradiol actions for the prevention of amhyloid-beta peptidel-40-induced toxicity in septal-derived cholinergic SN56 cells" Marin R., Guerra B., Morales A., Diaz M., Alonso R. J Neurochem 2003; 85; 1180-1189.

4- "Regulation of the membrane estrogen receptor-alpha: role of cell density, serum, cell passage number, and estradiol" Campbell C.H., Bulayeva N, Brown D.B., Gametchu B., Watson C.S. FASEB J 2002; 16(14); 1917-1927.

5- "Membrane-associated binding sites for estrogen contribute to growth regulation of human breast cancer cells" Marquez D.C., Pietras R.J. Oncogene 2001; 20(39); 5420-5430.

Another issue discussed was item 11 (35 USC 112, second paragraph). All parties agreed that the use of "previously" was indefinite and should be removed. The claims below reflect this issue.